

2nd PCR: Add overlaps to c3 and c6

	PCR 25 µL reaction (units : uL)	Final Concentration		Primers:			
5X Q5 reaction Buffer	5	1X		c3#1	f_Gbs_c3	r_Gbs_c7ADH1	
10 mM dNTPs	0,5	200 µM		c3#2	f_Gbs_c3	r_Gbs_c3c6	
10 µM Forward Primer	1,25	0.5 µM		c6	f_Gbs_c6	r_IDT_cir	
10 µM Reverse Primer	1,25	0.5 µM					
DNA (1 ng/µL)	1	<1 ng		c3_1, c3_2, c6 comes from 1st PCR, PCR Series 4			
DMSO (optional)	0	0		c3_0 and c3_3 comes from 8th PCR. PCR Series 4			
Q5 high fidelity DNA Polymerase (added last)	0,25	0.02 units/µl PCR					
Nuclease-free Water	15,75						
Total volume	25						
WORK ON ICE	c3#1 _ Q5						
number of reactions:	12		A1	c3_0#1		B1	c3_0#1
Mastermix	volume in uL		A2	c3_1#1		B2	c3_1#1
5X Q5 reaction Buffer	60		A3	c3_2#1		B3	c3_2#1
10 mM dNTPs	6		A4	c3_3#1		B4	c3_3#1
10 µM Forward primer	15	f_Gbs_c3	A5	c3#1 -		B5	c3#1 -
10 µM Reverse primer	15	r_Gbs_c7ADH1	A5'	c3#1 -		B5'	c3#1 -
DMSO (optional)	0		A6	c3_0#2		B6	c3_0#2
Q5 high fidelity DNA Polymerase (added last)	3		A7	c3_1#2		B7	c3_1#2
Nuclease-free Water	189		A8	c3_2#2		B8	c3_2#2
Total volume	288		A9	c3_3#2		B9	c3_3#2
volume of mastermix in each sample	24		A10	c3#2 -		B10	c3#2 -
			A10'	c3#2 -		B10'	c3#2 -
			A11	c6_0		B11	c6_0
WORK ON ICE	c3#2 _ Q5		A12	c6_1		B12	c6_1
number of reactions:	12		A13	c6_2		B13	c6_2
Mastermix	volume in uL		A14	c6_3		B14	c6_3
5X Q5 reaction Buffer	60		A15	c6 -		B15	c6 -
10 mM dNTPs	6		A15'	c6 -		B15'	c6 -
10 µM Forward primer	15	f_Gbs_c3	A -	no dna, no primer		B -	no dna, no primer
10 µM Reverse primer	15	r_Gbs_c3c6					
DMSO (optional)	0						
Q5 high fidelity DNA Polymerase (added last)	3						
Nuclease-free Water	189						

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Total volume	288							
WORK ON ICE	c6 _ Q5							
number of reactions:	12							
Mastermix	volume in uL							
5X Q5 reaction Buffer	60							
10 mM dNTPs	6							
10 µM Forward primer	15	f_Gbs_c3						
10 µM Reverse primer	15	r_IDT_cir						
DMSO (optional)	0							
Q5 high fidelity DNA Polymerase (added last)	3							
Nuclease-free Water	189							
Total volume	288							
WORK ON ICE	neg ctrl A- and B-							
number of reactions:	2							
Mastermix	volume in uL							
5X Q5 reaction Buffer	10							
10 mM dNTPs	1							
10 µM Forward primer	0							
10 µM Reverse primer	0							
DMSO (optional)	0							
Q5 high fidelity DNA Polymerase (added last)	0,5							
Nuclease-free Water	31,5							
Total volume	43							
Program A	GBS-Q5-A							
Initial Denaturation	98°C	30 seconds						
	98°C	10 seconds						
	64 °C	30 seconds						
35 Cycles	72°C	35 seconds						
Final Extension	72°C	2 minutes						
Hold	4 °C							

2nd PCR: Add overlaps to c3 and c6

Program B	GBS-Q5-B							
Initial Denaturation	98°C	30 seconds						
35 Cycles	98°C	5 seconds						
	63 °C	30 seconds						
	72°C	20 seconds						
Final Extension	72°C	2 minutes						
Hold	4 °C							